

**Effect of Dietary Protein and Metabolizable Energy Levels on Growth and Feed Utilization of Sea Bass (*Decentrarshus laborax*) Larvae**

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**ABSTRACT**

Three dietary protein levels (25, 35 and 45%) and two dietary metabolizable energy levels (250 and 300 kcal/100g diet) at a 3×2 factorial experiment were combined to study their effects on growth performance, body composition and feed utilization of sea bass (*Decentrarshus laborax*) larvae. The larvae had initial body weight of 0.8 g. In glass aquaria this experiment lasted for five weeks. Fish were fed 3 times daily to satiation 7 days per week with changing water daily by freshly stocked brackish water (15ppt) and continuous aeration. Fish were weighed every two weeks. Weight gain of sea bass increased significantly with increasing dietary crude protein level up to 45% and decreased significant with increasing energy levels from 250 and 300 kcal/100 g diets. Feed conversion ratio (FCR) improved with increasing dietary crude protein level up to 45% and no differences were found between 250 and 300 kcal/100g diets. Protein and lipid content of sea bass larvae increased significantly with increasing dietary crude protein level from 25% to 45%. Also, lipid content of sea bass larvae increased with increasing dietary energy level from 250 to 300 kcal/100 g diets, but, protein content decreased. The best SGR was observed with 45% dietary crude protein with 250 kcal/100g diet. Final body weight (FBW) of sea bass increased significantly ( $P<0.05$ ) with increasing dietary crude protein level up to 45% with 250 kcal/100 g diets. ER% increased significantly ( $P<0.05$ ) with increasing dietary crude protein level, but it decreased with increasing energy level. This result indicates that the best protein and energy levels for sea bass larvae (0.8 g BW) growth are 45% and 250 kcal, respectively. Lower protein level has given better protein utilization and a protein sparing effect but tended to result in reduced weight gain and feed intake, when compared with diet containing higher protein level at 45%.

**Keywords:** Sea bass (*Dicentrarchus labrax*), protein level, energy level.

**INTRODUCTION**

Fish culture in Egypt has developed into a major industry. Sea bass (*Dicentrarchus labrax*) is one of the most important commercial fish species in Egypt and it is commonly used in aquaculture (El-Shebly, 2009). The European sea bass (*Dicentrarchus labrax*, Linnaeus, 1758), being a member of the recently revised family of Moronidae. It was the first marine non

salmonid species to be commercially cultured in Europe and at present is the important commercial fish widely cultured in Mediterranean areas. Greece, Turkey, Italy, Spain, France and Egypt are now the biggest producers (Viale *et al.*, 2006).

In aquaculture, diet is often the single largest operating cost item and can represent over 50% of the operating costs in intensive aquaculture (El-Sayed, 1999; 2004). This cost

depends on many factors such as protein level, the source, and type of ingredients that could be derived from plant or animal resources, and manufacture practices (Glencross *et al.*, 2007). Nutrient and energy sources in feed are needed for the growth and maintenance of fish. Protein is probably the most important nutrient affecting fish growth and feed cost. Dietary energy level is also critical because protein source in the feed is utilized as an energy source when poor feed in energy is fed to fish, feed consumption decreases and resulted in growth reduction due to lack of other necessary nutrients for normal growth (Lovell, 1989 and El-Dahhar and Lovell, 1995). However, more studies are still needed on larval nutrient requirements (Planas and Cunha, 1998), since the maximum capacities of lipids and HUFA for fish larvae are not yet known. Lupatsch *et al.*, (2001, a, b and 2003) reported that successful fish culture depends on the supply of diets containing optimal levels of energy and nutrients for growth under any given condition. As feed is one of the principle costs in fish production, formulation must be based on sound knowledge of nutritional requirements for it to be economical (Sanver, 2005). Watanabe *et al.*, (1979); Nematipour *et al.*, (1992a) and Cowey, (1993) reported that the better feed utilization is not only the result of increased protein level, but also of improved protein quality. Protein quality is inter to energy utilization; therefore, replacing good quality dietary protein with lipid or carbohydrate might create a protein-sparing effect. The protein sparing effect of other energy sources has been demonstrated in a number of fish, like salmonids (Johnsen *et al.*, 1993 and Weatherup *et al.*, 1997), red sea bream (Takeuchi *et al.*, 1991), striped bass (Nematipour *et al.*, 1992b) and sea bream (Vergara *et al.*, 1996 and Company *et al.*, 1999a) by improvements in growth and protein efficiency.

One of the factors affecting dietary protein to energy ratios might be the use of fish of different weights, as protein

requirements decrease with increasing fish size (Page and Andrews, 1973; Kaushik and Luquet, 1984 and Masseret *et al.*, 1991). As protein is the main costly item for culture of this carnivorous marine fish; so, many researchers have attention in respect of protein requirement (Kikuchi *et al.*, 1992; Kim *et al.*, 2002 and Lee *et al.*, 2002), protein to energy ratio (Lee *et al.* 2000), fish meal replacement (Kikuchi *et al.*, 1994 and 2002) and dietary amino acid requirement study (Alamet *et al.*, 2000 and 2002).

Thus dietary protein requirements of European sea bass ranged from 42 to 52% depending on fish size, protein quality, protein to energy ratio and feeding management (Lim, 2003).

The aim of this study is to determine dietary crude protein and metabolizable energy requirements of sea bass (*Dicentrarchus labrax*) larvae.

## MATERIALS AND METHODS

This study was carried out in the Marine Fish Laboratory (MFL), Faculty of Agriculture (Saba-Basha); Alexandria University, Egypt.

### *Experimental procedure*

Fish were obtained in March from El-Meadia Fishing Port, the Mediterranean Sea. Fish were acclimated in glass aquaria for 15 days on the experimental diets and environmental conditions before experiment started. Aquaria contained sea water transferred from the sea (30 ppt) and with supplementary aeration continually. Fecal matter was removed by siphoning the water from the bottom of each aquarium one hour before giving the diet. All fish in each aquarium were weighed at the beginning of experiment and biweekly. Pooled sample of hundred fish of sea bass were killed at the beginning of the experiment and kept frozen for further chemical analysis. At the end of the experiment, 15 to 20 fish were taken randomly from each aquarium, killed and dried at 70°C for about 48 hours for final chemical analysis.

## PROTEIN AND ENERGY LEVELS ON GROWTH OF SEA BASS LARVAE

### *SEA WATER SUPPLY SYSTEM*

The sea water supply system consists of three components; the sea water supplies line, the sedimentation and disinfection facilities and the water storage tanks.

### *Diets formulation and preparation*

Diets were formulated from commercial ingredients of fish meal (FM), wheat flour, wheat bran, shrimp meal, soybean meal (SBM), yellow corn, vitamin and mineral mixture, fish oil, ascorbic acid and carboxy methyl cellulose (CMC). Diets composition and chemical analysis during the study are shown in Table (1). Diets were prepared as follow: Dry ingredients were passed through a sieve (0.6 mm diameter hole) before mixing into the diets. Oil was emulsified with equal amount of water using 0.7 % phosphatidyl choline (lecithin) according to El-Dhhar and El-Shazly (1993), and added to the diets of the experiments. Mixtures were homogenized in a feed mixer model SNFGA (Kitchen aid St. Joseph, M 149085 USA). Boiling water then blended to the mixtures at the rate of 50% for pelleting. An autoclave was used to heat the diets for 20 min after adding boiling water at a maximum pressure of 1.2 kg/ cm<sup>2</sup> G. Vitamins and minerals mixture were added to the diets after heat treatments. Aquaria management, heat treating of the diet and exogenous zymogene addition were made according to El-Dahhar (1999).

### *Experimental Design*

This experiment was conducted to evaluate the effect of combining three protein levels (25,35 and 45%) and two energy levels (250 and 300 kcal/100g) on growth performance, body composition and feed utilization of sea bass (*Dicentrarchus laborax*) larvae in glass aquaria using brackish water (15ppt) and temperature 26.3 °C ± 0.23 for 5 weeks. The three protein levels and two energy levels were evaluated in a factorial design with

three replicates for each treatment to be maintained in 18 glass aquaria. Sea bass larvae of initial body weight (BW) ± SE (0.8 ± 0.00) were stocked in glass aquaria at the rate of 20 fish per aquarium (40\* 60\*100cm). Fish were fed the pelleted diet three times daily at 9.00 am, 12.00pm and 16.00 pm at a satiation of feeding seven days per week. Fish were weighed every two weeks. Mortality was recorded daily.

### *Chemical and statistical analysis*

Crude protein (total-N x 6.25) and total lipid contents of the test diets and whole bodies were determine using the Kjeldahl method and ether-extraction method, respectively. Ash and moisture contents were analyzed following the Association of Official Analytical Chemists (AOAC 1995) using two replicate samples for each determination. Probabilities of P<0.05 were considered significant. s *et al.* 1979). The analysis of variance (ANOVA) tests were made according to Snedecor and Cochran (1981).

## RESULTS

### *Growth performance*

Survival, FBW and SGR of sea bass fed the six treatments are shown in Table 2. Survival of sea bass larvae increased significantly (P < 0.05) with increasing dietary crude protein level up to 45% (91.11% ± 0.39) and no significant difference between 35% and 45% crude proteins was found. Also, no significant differences (P > 0.05) were found between energy levels in survival rate.

FBW of sea bass larvae increased significantly (P < 0.05) with increasing dietary crude protein level up to 45% and decreased significant (P < 0.05) with increasing energy levels from 250 to 300 kcal. The highest FBW was observed with 45% dietary crude protein (3.32 ± 0.21g), followed by 35% (2.96 ± 0.05g) and 25% crude protein (2.22 ± 0.2g).

**Table (1): Composition and chemical analysis of the test diets used in the first experiment (feeding rates) different and in the second experiment, with protein and energy levels to feed sea bass (initial body weight 0.08 and 0.8g, respectively).**

Ingredients (%)	Protein level (%)					
	25		35		45	
	Metabolizable energy level (kcal/100g diet)					
	250	300	250	300	250	300
Wheat flour	27	20	10	6	5	0
Shrimp meal	10	14	12	14	25	25
Wheat bran	22.8	11.8	18.8	8.8	0	0
Soybean meal	8	8	15	15	20	20
Yellow corn	8	16	6	12	3.8	0.8
Fish meal	16	16	30	30	40	40
Fish oil	4	10	4	10	2	10
CMC <sup>1</sup>	3	3	3	3	3	3
Vit. & Min. mix <sup>2</sup>	0.8	0.8	0.8	0.8	0.8	0.8
Ascorbic acid	0.4	0.4	0.4	0.4	0.4	0.4
Proximate Analysis (%)						
Moisture	9.10	9.28	9.15	9.58	10.53	9.32
Crude protein	24.97	25.11	34.99	34.95	44.94	45.07
Crude lipid	10.50	17.44	8.50	15.3	6.8	12
Crude fiber	5.47	5.13	5.22	5.36	4.77	4.02
Carbohydrate (NFE) <sup>3</sup>	50.86	43.80	41.88	35.48	33.51	28.92
Crude ash	8.20	8.52	9.41	8.91	9.98	9.99
*Gross Energy (GE) and (ME) kcal/100g diet	411.52 (250)	448.21 (300)	412.21 (250)	448.26 (300)	412.48 (250)	448.41 (300)

*1-carboxy methyl cellulose.*

*2-vitamin and mineral mixture/kg premix: vitamin A, 4.8 million IU; D 3, 0.8 million IU; E 4g; K, 0.8g; B1 0.4g; riboflavin, 1.6g; B6, 0.6g; B12, 4mg; pantothenic acid, 4g; nicotinic acid, 8g; folic acid, 0.4g; biotin, 20 mg; choline chloride, 200 g; Cu, 4g; I, 0.4g; Iron, 12g; Mn, 22g; Zn, 22 g; selenium, 0.4 g.*

*3-NFE is nitrogen free extract = 100-(cp+cl+cf+ash).*

- 2% Zymogene® was added to each diet according to El-Dahar (1999a)
- \* gross energy (ge) content was calculated by using the factors 5.65, 9.4 and 4.1 kcal/g for protein, ether extract and carbohydrate, respectively (Jobling, 1983).

## PROTEIN AND ENERGY LEVELS ON GROWTH OF SEA BASS LARVAE

But, it decreased from  $2.79 \pm 0.49$  g when the fish fed diet containing 300 kcal ME/100g to  $2.69 \pm 0.72$ g with 250 kcal diet (Table 2). SGR of sea bass larvae fed the test diets had the same trend like FBW, since it increased significantly ( $P < 0.05$ ) with increasing dietary crude protein level up to 45%, and decreased significant ( $P < 0.05$ ) with increasing energy level ( $P < 0.05$ ) (Table 2). The highest SGR was observed with 45% dietary crude protein ( $4.06 \pm 0.18\%$  /d), followed by 35% ( $3.47 \pm 0.06\%$  /d) and 25% crude protein ( $2.91 \pm 0.47\%$ /d). On the other hand, increasing the energy level from 250 to 300 kcal did not affect any additional increase in FBW, survival and SGR. The interaction between the two factors (dietary crude protein level and energy level) was also significant ( $P < 0.05$ ) for FBW and SGR. But it was not significant for survival ( $P > 0.05$ ). FBW of sea bass fed at three dietary crude protein levels 25, 35 and 45 % each with two ME levels 250 and 300 kcal were found to be ( $2.24 \pm 0.02$ ;  $2.19 \pm 0.03$ ;  $2.72 \pm 0.03$ ;  $2.67 \pm 0.01$ ;  $3.42 \pm 0.01$  and  $3.21 \pm 0.04$  g/ fish) for the fish fed the diets in (T1, T2, T3, T4, T5, T6), respectively. The best FBW was found to be  $3.42 \pm 0.01$  g for the fish fed at (T5) 45. The highest SGR was also observed at 45% dietary crude protein level with 250 kcal/100g diet (T5).

Feed conversion ratio (FCR) of sea bass fry improved with increasing dietary crude protein level from 25% to 45%. It was found to have the values of ( $1.919 \pm 0.028$  and  $1.293 \pm 0.011$  respectively). However, there are no significant differences ( $P > 0.05$ ) in FCR between the fish groups fed energy levels at 250 and 300 kcal (Table 3). Also, interaction between protein level and energy level was significant ( $P < 0.05$ ) for weight gain and feed consumption. The best feed consumption, gain and FCR of sea bass fed at three dietary crude protein levels (25, 35 and 45%) and two energy levels (250 and 300 kcal/100g diet) were found to be ( $3.36 \pm 0.014$ ;  $2.62 \pm 0.01$  and  $1.283 \pm 0.009$  g/fish), respectively for the fish fed

at 45% dietary crude protein level with 250 kcal/100g diet energy level (T5). Weight gain increased significantly ( $P < 0.05$ ) with increasing dietary protein level up to 45% with 250 kcal/100g diet (T5). Also, feed consumption increased significantly ( $P < 0.05$ ) with increasing dietary protein level up to 45 % with 250 kcal/100g diet (T5) (Table 3).

### Body composition

Table (4) shows that body composition of sea bass (*Decentrarshus laborax*) larvae was affected by dietary protein levels and two energy levels. Moisture content of sea bass decreased with increasing dietary crude protein level from 25 to 45%. The highest moisture contents was found to be ( $75.19 \pm 0.007\%$ ) for the fish maintained at 25% protein level, while the lowest was ( $72.09 \pm 0.005\%$ ) for 45% dietary protein level. Also moisture content of fry increased significantly ( $p < 0.05$ ) with increasing energy level from 250 to 300 kcal/100 g diets. The highest moisture contents were found to be ( $73.86 \pm 0.006\%$ ) for the fish maintained at 300kcal energy level, while the least was ( $73.29 \pm 0.009\%$ ) for 250 kcal energy level.

Protein content of sea bass increased significantly ( $P < 0.05$ ) with increasing dietary protein level from 25 to 45%. The highest protein content was found to be ( $15.67 \pm 0.58\%$ ) for the fish maintained at 45 %. On the other hand, protein content of larvae decreased significantly ( $P < 0.05$ ) with increasing energy level from 250 to 300 kcal / 100 g diet. There was a significant ( $P < 0.05$ ) effect of protein levels and energy levels on protein content of the fish body.

Also, lipid contents increased significantly ( $P < 0.05$ ) with increasing protein and energy levels (Table 4). It was found to be ( $3.16 \pm 0.037$ ;  $4.17 \pm 1.387$  and  $5.36 \pm 0.189\%$ ) and ( $3.97 \pm 1.592$  and  $4.49 \pm 1.697\%$ , respectively).

Table (2): Mean  $\pm$  standard error (SE) of survival, final body weight and specific growth rate (SGR) of sea bass (*Decentrarshus laborax*) (0.8 g initial BW) fed at three dietary protein levels (25, 35 and 45%) with two energy levels (250 and 300 kcal/100g diet) for five weeks.

Protein Level	Energy Level	Treatment	Survival	Final body Weight	SGR
%	kcal/100g feed		%	(g/fish)	% /d
25%	250	T1	82.22b $\pm$ 0.41	2.24d $\pm$ 0.02	2.94d $\pm$ 0.014
	300	T2	82.22b $\pm$ 0.41	2.19d $\pm$ 0.03	2.89e $\pm$ 0.019
35%	250	T3	84.44ab $\pm$ 0.41	2.72c $\pm$ 0.03	3.40c $\pm$ 0.024
	300	T4	86.66ab $\pm$ 0.41	2.67c $\pm$ 0.01	3.44c $\pm$ 0.030
45%	250	T5	91.11a $\pm$ 0.39	3.42a $\pm$ 0.01	4.15a $\pm$ 0.004
	300	T6	91.11a $\pm$ 0.39	3.21b $\pm$ 0.04	3.97b $\pm$ 0.018
<b>poold means</b>					
25%			82.22h $\pm$ 0.41	2.22k $\pm$ 0.04	2.91k $\pm$ 0.047
35%			85.55h $\pm$ 0.41	2.96h $\pm$ 0.05	3.47h $\pm$ 0.06
45%			91.11g $\pm$ 0.39	3.32g $\pm$ 0.21	4.06g $\pm$ 0.18
	250		86.66x $\pm$ 0.40	2.79x $\pm$ 0.49	3.53x $\pm$ 0.86
	300		87.73x $\pm$ 0.40	2.69y $\pm$ 0.72	3.43y $\pm$ 0.76

\* Means in the same column not sharing the same letter are significantly different  $P < 0.05$ .

SGR = 100 (ln final BW - ln initial BW) / days

Table (3). Mean  $\pm$  standard error (SE) of feed consumption, weight gain and feed conversion ratio (FCR) of sea bass (*Decentrarshus laborax*) (0.8 g initial BW) fed at three dietary protein levels (25, 35 and 45%) with two energy levels (250 and 300kcal/100g diet) for five weeks.

Protein Level	Energy Level	Treatment	Feed Consumption	Weight Gain	FCR
%	kcal/100g feed		(g/fish)	(g/fish)	
25%	250	T1	2.78e $\pm$ 0.67	1.44d $\pm$ 0.02	1.931a $\pm$ 0.073
	300	T2	2.66f $\pm$ 0.014	1.39e $\pm$ 0.03	1.913a $\pm$ 0.039
35%	250	T3	3.07d $\pm$ 0.106	1.92c $\pm$ 0.03	1.599b $\pm$ 0.073
	300	T4	2.99c $\pm$ 0.071	1.87d $\pm$ 0.01	1.599 b $\pm$ 0.084
45%	250	T5	3.36a $\pm$ 0.014	2.62a $\pm$ 0.01	1.283d $\pm$ 0.009
	300	T6	3.15b $\pm$ 0.014	2.41b $\pm$ 0.04	1.307d $\pm$ 0.018
<b>Poold means</b>					
25%			2.72k $\pm$ 0.12	1.42k $\pm$ 0.05	1.915h $\pm$ 0.028
35%			3.03j $\pm$ 0.15	1.89j $\pm$ 0.05	1.603 j $\pm$ 0.037
45%			3.26h $\pm$ 0.09	2.52h $\pm$ 0.21	1.293k $\pm$ 0.011
	250		3.07x $\pm$ 0.41	1.99x $\pm$ 0.84	1.543 x $\pm$ 0.405
	300		2.93y $\pm$ 0.57	1.89y $\pm$ 0.59	1.550 x $\pm$ 0.375

\* Means in the same column not sharing the same letter are significantly different  $P < 0.05$ .

\*FCR = (Feed/gain).

## PROTEIN AND ENERGY LEVELS ON GROWTH OF SEA BASS LARVAE

Table (4). Mean  $\pm$  standard error (SE) of moisture, protein and lipid content dry matter basis in the carcass of sea bass (*Decentrarshu laborax*) (0.8 g initial weight) fed at three dietary protein levels (25,35 and 45%) with two energy levels (250 and 300 kcal) for five weeks.

Protein Level	Energy Level	Treatment	Moisture %	Protein %	Lipid %
%	kcal/100g feed				
Initial			77.64 $\pm$ 0.006	14.36 $\pm$ 0.015	1.922 $\pm$ 0.003
25%	250	T1	75.13a $\pm$ 0.002	14.77e $\pm$ 0.008	3.18e $\pm$ 0.007
	300	T2	75.26a $\pm$ 0.001	14.14f $\pm$ 0.024	3.14e $\pm$ 0.06
35%	250	T3	73.15c $\pm$ 0.006	15.60b $\pm$ 0.022	3.48d $\pm$ 0.005
	300	T4	73.73b $\pm$ 0.001	14.91d $\pm$ 0.037	4.87c $\pm$ 0.011
45%	250	T5	71.59e $\pm$ 0.009	15.96a $\pm$ 0.014	5.26b $\pm$ 0.021
	300	T6	72.59d $\pm$ 0.006	15.38c $\pm$ 0.014	5.45a $\pm$ 0.032
<b>Pool means</b>					
25%			75.19h $\pm$ 0.007	14.45k $\pm$ 0.627	3.16k $\pm$ 0.037
35%			73.44j $\pm$ 0.003	15.26j $\pm$ 0.693	4.17j $\pm$ 1.387
45%			72.09k $\pm$ 0.005	15.67h $\pm$ 0.58	5.36h $\pm$ 0.189
	250		73.29y $\pm$ 0.009	15.44x $\pm$ 2.267	3.97y $\pm$ 1.592
	300		73.86x $\pm$ 0.006	14.81y $\pm$ 2.294	4.49x $\pm$ 1.697

\* Means in the same column not sharing the same letter are significantly different  $P < 0.05$ .

Also, interaction between dietary protein levels and energy levels was highly significant ( $P < 0.05$ ) Table (4). The lowest moisture content of sea bass was found at 250 kcal energy level and 45% crude protein (T5). The highest protein content was found for sea bass received 45% dietary protein level and 250 kcal level (T5). While the least protein content was found with larvae received diet containing 25% protein level with 300 kcal energy level (T2). Larvae fed diets containing 45% protein level with 250 and 300 kcal energy levels exhibited lipid contents of (5.26  $\pm$  0.021) and (5.45  $\pm$  0.032%), (T5 and T6, respectively). They were significantly higher ( $P < 0.05$ ) than that of sea bass larvae fed 25% and 35% protein levels with both energy levels. Also, the interaction between dietary protein levels and energy levels was significant ( $P < 0.05$ ) for lipid and protein content (Table 4).

### Protein and energy utilization

PER (body weight gain/protein fed) of sea bass decreased significantly ( $P < 0.05$ ) as

crude protein level increased from 25 to 45% (2.09  $\pm$  0.03, 1.79  $\pm$  0.32 and 1.72  $\pm$  0.34). But, PER was not affected significantly ( $P > 0.05$ ) as energy increased from 250 to 300 kcal (Table 5).

On the other hand, interaction between dietary crude protein and energy levels was found to be not significant ( $P > 0.05$ ) for PER, and PPV%, but ER% was found to be significant ( $P < 0.05$ ) (Table 5).

ER% (100 retained energy/energy fed) significantly increased by increasing crude protein level in the diet. Also, ER decreased significantly ( $P < 0.05$ ) as energy level increased from 250 to 300kcal (35.35  $\pm$  0.183 and 29.76  $\pm$  0.156). The highest ER% value was obtained at 45% crude protein level (43.38  $\pm$  0.079) and the lowest was obtained at 25% (22.85  $\pm$  0.039). The best ER of sea bass fed at three dietary protein levels (25, 35 and 45%) and two energy levels (250 and 300 kcal) was found with the group maintained at 45% dietary crude protein with 250 kcal (47.86  $\pm$  0.003%) (T5) (Table 5).

Table (5). Mean  $\pm$  standard error (SE) of energy retention (ER %), protein productive value (PPV %) and protein efficiency ratio (PER) of sea bass (*Decentrarchus laborax*) (0.8 g initial weight) fed at three dietary protein levels (25, 35 and 45%) with two energy levels (250 and 300 kcal).

Protein Level	Energy Level	Treatment	ER%	PPV%	PER
%	kcal/100g feed				
25%	250	T1	25.33e $\pm$ 0.012	31.04a $\pm$ 0.99	2.07b $\pm$ 0.08
	300	T2	20.37f $\pm$ 0.006	29.43b $\pm$ 0.53	2.10a $\pm$ 0.04
35%	250	T3	32.86c $\pm$ 0.002	28.86c $\pm$ 1.21b	1.79c $\pm$ 0.08
	300	T4	30.03d $\pm$ 0.014	27.03d $\pm$ 1.45	1.78c $\pm$ 0.09
45%	250	T5	47.86a $\pm$ 0.003	28.49c $\pm$ 0.175	1.73d $\pm$ 0.01
	300	T6	38.89b $\pm$ 0.013	26.72d $\pm$ 0.502	1.70d $\pm$ 0.02
<b>Pooled means</b>					
25%			22.85k $\pm$ 0.039	30.24g $\pm$ 0.102	2.09g $\pm$ 0.03
35%			31.45j $\pm$ 0.069	27.95h $\pm$ 3.643	1.79h $\pm$ 0.32
45%			43.38i $\pm$ 0.079	27.61h $\pm$ 3.223	1.72k $\pm$ 0.34
	250		35.35x $\pm$ 0.183	29.47x $\pm$ 0.384	1.86x $\pm$ 0.04
	300		29.76y $\pm$ 0.156	27.73y $\pm$ 0.430	1.86x $\pm$ 0.01

\* Means in the same column not sharing the same letter are significantly different  $P < 0.05$ .

\*ER% = 100 (gained Energy/Energy fed)

\*PPV% = 100 gained protein/protein fed.

\*PER = gain/protein fed

PPV% (100 gained protein/protein fed) of sea bass decreased significantly ( $P < 0.05$ ) as crude protein levels increased from 25 to 45 % (30.24 $\pm$ 0.102, 27.95 $\pm$ 3.64 and 27.61  $\pm$ 3.22%). Also, PPV decreased significantly ( $P < 0.05$ ) as energy level increased from 250 to 300kcal (29.47  $\pm$  0.384 and 27.73  $\pm$  0.43%) (Table 5).

## DISCUSSION

This study was carried out to investigate the effect of different dietary crude protein and energy levels feed utilization and body composition of sea bass (*Dicentrarchus labrax*). Despite of the commercial importance and rapid expansion of sea bass aquaculture, very little is known about the nutritional requirements of this species. However, based on the information available, nutritional requirements of fish do not vary greatly among species

(Lovell). Currently, little information exists regarding the nutritional demands of fry sea bass, studies have been conducted with other carnivorous marine species such as mutton snapper, *Lutjanus analis* (Watanabe *et al.*, 2001); drum, *Nibea miichthioides* (Wang *et al.*, 2006a and b); black sea bass, *Centropristis striata* (Rezek *et al.*, 2005 and 2007) (Thoman *et al.*, 1999); sea bass, family Serranidae (Borlongan and Parazo, 1991; Catacutan and Coloso, 1995; Company *et al.*, 1999a and b; Russel *et al.*, 1986; and Lanari *et al.*, 1999 and 2002; Perez *et al.* 1997 and Perez and Oliva - Teles 2005 and 2006); Japanese flounder, (*Paralichthys olivaceus* (Kikuchi, 1999); yellowtail, *Seriola quinqueradiata* (Masumoto *et al.*, 1996) and hybrid striped bass, *Morone* sp. (Nematipour *et al.*, 1992a and b and Gaylord and Gatlin, 2000). In the present study, the FBW



## PROTEIN AND ENERGY LEVELS ON GROWTH OF SEA BASS LARVAE

and SGR of sea bass with an initial of 0.8 g were increased significantly ( $P < 0.05$ ) with increasing dietary crude protein up to 45% with 250 kcal / 100g feed. Also, the WG and survival rate of sea bass tended to improve with increasing dietary crude protein level up to 45%. There were significant differences ( $P < 0.05$ ) in WG with increasing dietary crude protein level. These results are similar to the findings of Perez *et al.* (1997), they reported that the best growth for sea bass fingerlings was related to a diet containing 45% CP.

Little nutritional data exists regarding levels of crude protein and lipid necessary to promote growth in larvae sea bass. Catacutan *et al.* (2001) has indicated that crude protein levels for optimum growth of laboratory reared mangrove red snapper is greater than 40%. Fish fed diets containing 42.5% crude protein showed significantly higher growth rates than those fed diets with 35% crude protein. However, no significant differences in growth were observed between fish fed diets containing 42.5 and 50% crude protein. Although little data exists in regards to the dietary requirements of sea bass, studies have been conducted with other carnivorous marine species.

Also, Thoman *et al.* (1999) found that red drum require crude protein levels of 44% to achieve optimum growth. Weight gain and FE were found to increase with protein and energy contents of the diets. An increase of lipid content in diets containing 44% protein resulted in increased FE, but also significantly increased interapritoneal fat ratio (IPFR). Red drum fed diets containing 40% protein was found to optimize growth, feed conversion ratio (FCR), and survival at dietary lipid levels of 7.4 and 11.2%. Fish fed a diet containing 18.8% lipid were found to have lower weight gains and higher feed conversion ratios than fish fed lower levels of lipid (Williams and Robinson, 1988).

Lim (2003) reported that dietary protein requirements of European sea bass ranged from 42 to 52 % depending on fish size, protein

quality, and protein to energy ratio and feeding management. Also, the protein requirement for maximum growth of juvenile sea bass has been estimated to be around 50% (Hidalgo and Alliot, 1988; Barnabe, 1990; Ballestrazzi *et al.*, 1994 and Wilson, 2002). Several studies with different species of the Serranidae family of sea bass indicate that optimum growth, FE, and protein efficiency (PE) are achieved when dietary crude protein is between 42.5 and 48 %, while in conjunction with dietary lipid concentration of 10-14% (Borlongan and Parazo, 1991; Catacutan and Coloso, 1995; Shiau and Lan, 1996 and Perez *et al.* 1997). Maximum growth in laboratory reared mutton snapper, *Lutjanu analis*, occurred when fish were fed a ration containing 45% crude protein in conjunction with 6-9% crude lipid (Watanabe *et al.* 2001). Whereas, Boonyaratpalin (1991) reported that the protein content of sea bass diets could be reduced from 50 % to 45 % without affecting the growth when the lipid level was increased from 15 % to 18%. Catacutan and Coloso (1995) found that a dietary lipid content of 9.3 % was optimum for sea bass if a 43 % crude protein level was used.

In the present study, the best FCR of sea bass with an initial of BW 0.8 g was obtained at 45% crude protein with 250 kcal / 100g feed. FCR was found to decrease with increasing the dietary crude protein level (Table.3). These results are in agreement with those reported by Shiau *et al.* (1989) for hybrid Tilapia (*O. niloticus* x *O. aureus*) reared in seawater. In the present study, specific growth rate (SGR) was found to increase with increasing dietary protein level (Table, 3). This result agrees with what of Siddiqui *et al.* (1988). On the other hand, Satpathy and Ray (2009) reported that The best performance of rohu, *Labeo rohita* in terms of weight gain (%), specific growth rate (SGR; % per day), and feed conversion ratio (FCR) was recorded with diet containing 40% protein and 35% dextrin as a source of dietary carbohydrate.

In the present study, feed consumption decreased with increasing energy level from 250 to 300 kcal/100g diet. This is probably due to a faster absorption of nutrients when energy level is lower. These results are similar to the findings of Lupatsch *et al.* (2001a and b), they found a tendency for a decrease in feed intake by sea bass and sea bream fed to satiation when dietary DE level increased. The energy content of the feed is considered to be the main factor controlling feed consumption in finfish (Jobling and Wandsvik, 1983; Kaushik and Luquet, 1984; Kaushik and Oliva-Teles, 1985; Boujard and Medale, 1994 and Paspatis and Boujard, 1996). In the work of Lanari *et al.* (2002), they reported growth performance and voluntary feed intakes (VFI) of sea bass of different weights reared under various water temperatures. Grisdhale-Helland and Helland (1997) found an increase in feed intake in response to increasing dietary protein and decreasing carbohydrate levels in salmon fed every 5 min during the light period.

However, more studies are still needed on larval nutrient requirements (Planas and Cunha, 1998), since the maximum capacities of lipid and HUFA for fish larvae are not yet known. Criteria used for evaluating optimal DP/DE ratios are the maximum weight and protein deposition and the highest protein and energy efficiencies (Kaushik and Medale, 1994). In the European sea bass (*Dicentrarchus labrax*), a diet containing 19% lipid increased fish growth compared to diets with 11% and 15% lipid content (Lanari *et al.* 1999). However, the effect of dietary lipid level is different at various fish developmental stages. However, the growth rates of larval sea bass fed diets of 12% to 20% lipid content did not differ (Salhi *et al.*, 1994).

In our study, sea bass responded better on the low energy 250 kcal/100-g diets than high energy 300 kcal/100-g diets at 45% crude protein when it was fed to satiety three times daily. These results are in agreement with Lee *et*

*al.*, (2000) which showed that juvenile flounder grew better on the low energy diet (303 kcal/100-g diets) than on the high energy diet (411 kcal / 100-g diets) at 48 % protein level when it was satiety twice daily or three times daily; however, fish responded better on the high energy diet when it was fed to satiety once daily.

In our study, the results of this trial indicate that an increase of energy level from 250 to 300 kcal/100g diet did not improve growth performance and feed efficiency of sea bass fry. The increase of energy level had no protein sparing effect. These results agree with the results of Peres and Oliva-Teles, (1999a and b) they found no protein sparing effect for European sea bass juveniles (*Dicentrarchus labrax* IBW=7 g). Moreover some other researchers did not find any protein sparing effect in sea bass and sea bream (Lanari *et al.*, 1998; and Company *et al.*, 1999b).

Whereas, Page and Andrews (1973) and Helland and Grisdale-Helland (1998) observed that better growth or feed efficiency of fish fed high energy diet than low energy diet, called protein -sparing effect. Only a few studies have been carried out to determine the optimal energy and protein requirements of marine fish using high-energy diets (Curry Woods III *et al.*, 1995; Guinea and Fernandez, 1997; Lupatsch and Kissil, 1998 and Lupatsch *et al.*, 2001b).

But, Watanabe *et al.* (2001) found that at a dietary protein level of 45%, juvenile mutton snapper displayed optimum growth at the lowest levels of inclusion of dietary lipids. On the other hand, according to Santinha *et al.* (1999) when diets contained 15% lipid, increasing protein level from 47 to 51% led to an improvement in feed efficiency in sea bream (*Sparus aurata*) but there was no effect of protein level in 21% lipid diets. But, Catacutan and Coloso (1995) found that at a dietary protein level of 42.5% and 10% lipid with P/E ratio of 128 mg protein/kcal was found to be optimum for juvenile sea bass (body weight,  $1.34 \pm 0.01$  g).

## PROTEIN AND ENERGY LEVELS ON GROWTH OF SEA BASS LARVAE

In the present study, sea bass fed the high energy diet contained higher body lipid than low energy diet at all protein levels without improvement of growth. This result agrees with the finding of Boujard *et al.*, (2004) who showed that, increasing the dietary lipid level led to a significant decrease in voluntary feed intake without affecting growth rate. There was a significant and inverse effect of the dietary fat content on whole body moisture and fat levels, with highest lipid and lowest moisture contents in sea bass fed diet containing the highest lipid level; muscle lipid concentration was however not affected. Also, high dietary energy level increased body lipid without improvement of growth performance in several species of fish (Marais and Kissil, 1979; Lie *et al.*, 1988; El-Dahhar and Lovell, 1995 and Peres and Oliva-Teles, 1999a and b). High dietary energy levels must be carefully evaluated as it may negatively affect carcass composition, mainly due to an increase of lipid deposition (Hillestad and Johnsen, 1994). On the other hand, the inclusion of 30% of dietary lipid did not modify growth rate but reduced protein and energy retention in *Dicentrarchus labrax* (Peres and Oliva-Teles, 1999a and b), indicating that the common practice to administer an identical commercial feed both for sea bass and Sparidae should be carefully considered when a high lipid level diet is used. Lanari *et al.* (1999) suggested that protein and ash in sea bass *Dicentrarchus labrax* seems more life-cycle and size dependent, while the fat of fish increases with energy intake and fish size.

In our study, lower protein level has given better protein utilization and a protein sparing effect but reduced weight gain and feed intake, when compared with diet containing higher protein levels at 45%. With respect to diet nutritional utilization, PER and PPV were significantly higher ( $P < 0.01$ ) in fish fed the diet with low protein content, indicating that the diet with 25 % of protein was found better utilized by fry sea bass under the present experimental conditions. In the present study, the high energy

level in the diets could have got a sparing effect of proteins, which was more evident in fish, fed the lower protein level at 25%. This result agrees with the finding of (Bonaldo *et al.*, 2004), they showed that juvenile sharpsnout sea bream consumed a higher amount of food when fed a 52.5% protein and 9.0% carbohydrate diet. This is probably due to a faster absorption of nutrients when carbohydrate level is lower. On the other hand, the 46.7% protein diet showed statistically higher, GPE and PER than 52.5% protein diet, giving a protein sparing effect. Also, the PER was decreased with increasing dietary protein above the optimal level 45%. These agree with work previously reported (Lochmann and Phillips, 1994).

Also, the obtained results are lower than PER observed for Nile tilapia fed graded protein level from 12 to 32% dietary crude protein levels (El-Dahhar., 1999b) and for mullet fed crude protein 41% (Abdallah., 2001) and for Clarias catfish fed 20 – 40% dietary crude protein levels (Jantrarotai *et al.*, 1998). Therefore, sea bass seems likely to utilize protein for body growth less efficiently than do mullet, rainbow trout, Nile tilapia and Clarias catfish.

Requirements of protein for maximum growth in teleost larval and juvenile stages are nearly twice as high as in older fish (Dabrowski, 1986). The reason for this may be due to underestimated IDAA requirements because of suboptimal growth rates in juvenile fish when purified diets were used (Dabrowski, 1986 and NRC, 1993). This result indicates that the best protein level and energy level for sea bass (0.8 g BW) is 45% and 250 kcal, respectively in terms of total weight gain. Lower protein level has given better protein utilization and a protein sparing effect but tended to result in reduced weight gain and feed intake, when compared with diet containing higher protein levels at 45%.

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## PROTEIN AND ENERGY LEVELS ON GROWTH OF SEA BASS LARVAE

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## تأثير مستوى البروتين والطاقة الميتابولزمية على النمو والاستفادة من الغذاء ليرقات أسماك القاروص

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أجريت هذه الدراسة في كلية الزراعة ( سابا باشا ) جامعة الإسكندرية ، حيث أجريت التجربة بهدف دراسة تأثير كل من مستويات البروتين الخام ومستويات الطاقة، على كفاءة النمو والاستفادة من الغذاء على أسماك القاروص في مراحل النمو الأولى.

الهدف من إجراء هذه التجربة هو دراسة تأثير مستويات مختلفة من البروتين الخام ومستويات من الطاقة على كفاءة النمو والاستفادة من الغذاء لأسماك القاروص في مراحل النمو الأولى في تجربة عاملية (3×2) باستخدام 3 مستويات من البروتين الخام (25 ، 35 ، 45%) مع مستويين من الطاقة الميتابولزمية (250 ، 300 ك كالورى/ 100 جم علف) . ، بمعدل تخزين 20 سمكة بكل حوض وقد استمرت التجربة 5 أسابيع بوزن ابتدائي (0.8 جم ) ، وتتم التغذية حتى الشبع 7 أيام في الأسبوع وتقدم العلائق 3 مرات يوميا ويتم وزن الأسماك كل اسبوعين .

وقد أظهرت النتائج ما يلي :

أفضل معدلات نمو تم الحصول عليها عند مستوى بروتين 45% مع مستوى طاقة (250 كيلوكالورى). وقد وجد أن معدل نمو الاسماك يزداد بزيادة مستويات البروتين حتى 45% ولا يوجد اختلاف معنوي بين مستويات الطاقة. لوحظ زيادة معدل النمو النوعي للغذاء وأيضا الوزن النهائى بزيادة مستويات البروتين حتى 45% مع مستوى الطاقة 250 ك كالورى ،وبقياس التداخل في التأثير وجد أن له تأثير معنوي عند مستوي (0.05) ووجد أن معامل تحويل الغذاء تحسن بزيادة مستوى البروتين حتى 45% ولا يوجد اختلاف معنوي عند زيادة مستوى الطاقة.

وقد وجد أيضا أن محتوى أسماك القاروص من الدهون والبروتين قد تأثر بزيادة مستوى البروتين ،وأیضا هناك تأثير معنوي بزيادة مستوى الطاقة حتى 300 ك كالورى على محتوى جسم السمكة من الدهن مع انخفاض فى محتواها من البروتين لوحظ ارتفاع الطاقة المحتجزة بزيادة مستويات البروتين حتى 45% مع مستوى الطاقة 250 ك كالورى ،وبقياس التداخل في التأثير وجد أن له تأثير معنوي عند مستوي (0.05) .

ومما سبق نستنتج أن زيادة مستويات البروتين حتى 45% مع مستوى طاقة 250 ك كالورى ، أدى إلى تحسن معدلات النمو والاستفادة من الغذاء وأيضا معدل النمو النوعي ،كما ارتفع معدل الاعاشة بزيادة مستوى البروتين ،ولا يوجد اختلاف معنوي بزيادة مستوى الطاقة،كما ارتفعت الطاقة المحتجزة. ومن هذه البيانات اتضح أن أفضل مستوى من البروتين الخام هو 45% مع 250 ك كالورى وهى المستويات المثلى التي حققت أعلى معدل نمو لأسماك القاروص عند وزن ابتدائي (0.8 جم).